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# Ecotoxic effects of loratadine and its metabolic and light-induced derivatives

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## Abstract

Loratadine and desloratadine are second-generation antihistaminic drugs. Because of human administration, they are continuously released *via excreta* into wastewater treatment plants and occur in surface waters as residues and transformation products (TPs). Loratadine and desloratadine residues have been found at very low concentrations (ng/L) in the aquatic environment but their toxic effects are still not well known. Both drugs are light-sensitive even under environmentally simulated conditions and some of the photoproducts have been isolated and characterized. The aim of the present study was to investigate the acute and chronic ecotoxicity of loratadine, desloratadine and their light-induced transformation products in organisms of the aquatic trophic chain. Bioassays were performed in the alga *Pseudokirchneriella subcapitata*, the rotifer *Brachionus calyciflorus* and in two crustaceans, *Thamnocephalus platyurus* and *Ceriodaphnia dubia*. Loratadine exerted its acute and chronic toxicity especially on *Ceriodaphnia dubia* (LC50: 600 µg/L, EC50: 28.14 µg/L) while desloratadine showed similar acute toxicity among the organisms tested and it was more chronically effective compound in *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*. Generally, transformation products were less active both in acute and chronic assays.

**Keywords:** antihistaminic drug; loratadine; desloratadine; acute toxicity; chronic toxicity; photoproducts.

## Highlights

- Loratadine was irradiated by UVB and sunlight and its photoproducts were isolated and characterized.
- Loratadine, desloratadine and light-induced TPs were tested in aquatic organisms.
- Generally, transformation products were less active both in acute and chronic assays.
- Desloratadine was the most chronically effective compound in *C. dubia* and *P. subcapitata*.

## 1. Introduction

Drugs are continuously released as mixtures of parent compounds and metabolites and enter the aquatic environment through hospital and municipal wastewaters. Here, these mixtures may undergo transformations due to redox or light-induced reactions, hydrolysis, and other reactions leading to transformation products, in some cases more harmful than parent compounds (DellaGreca et al., 2014; Passananti et al., 2015; Isidori et al., 2016). The importance of these events in the breakdown of drugs has stimulated a large number of researches concerning kinetics, degradation mechanism, isolation and toxicity of the transformation products (Lambropoulou and Nollet, 2014). Generally, the most commonly occurring drugs in the aquatic systems are the most administered. However, some classes of drugs highly utilized by patients are not detected in the waters because rapidly degraded, while in some cases drugs less utilized are detected at high concentrations because resistant to biodegradation. Among the most administered drugs, antihistamines are detected in surface waters because of their poor removal by conventional wastewater treatments (Kosonen and Kronberg, 2009; Radjenovic' et al., 2009; Valcarcel et al., 2011) and due to their low polarity and scarce volatility, they may represent a hazard for the aquatic ecosystem (Berninger and Brooks, 2010; Kristofco and Brooks, 2017).

**Among antihistamines**, ranitidine, difenidramine, cimetidine and loratadine are the most detected in the effluents of sewage treatment plants and the detection of loratadine in surface waters has exceeded therapeutic hazard values (THVs) showing the need of understanding the aquatic toxicology, hazards and risks associated with this drug (Kristofco and Brooks, 2017). Loratadine is a second-generation antihistaminic drug so called because it causes less sedation and drowsiness than the first-generation antihistamines used to treat allergic reactions, approved by US Food and Drug Administration in 1993. Loratadine is a selective inverse agonist of peripheral H<sub>1</sub>-receptors (Witiak, 1970, Peyrovi and Hadjmohammadi, 2015). It is mainly metabolized through the hepatic system to desloratadine, which is a pharmacologically active compound, deriving from the loss of carbamate moiety (Yumibe et al., 1996). **Forty percent and 42% of the ingested loratadine dose is excreted unchanged in urine and the feces, respectively** (Ramanathan et al., 2007). It has been detected in surface waters in Europe (in some Spanish river samples) in the low concentration range of 3.96-17.1 ng/L (Lopez-Serna et al., 2012) but also in wastewater effluents in Europe, North-America and Asia-Pacific with a maximum concentration of 58.5 ng/L (Kristofco and Brooks, 2017). Desloratadine has been detected in Europe with a maximum concentration of 81 ng/L (Kristofco and Brooks, 2017). Both drugs have also been recovered in lower amounts in marine water of Mediterranean coasts (Moreno-Gonzalez et al., 2015). Based on the antihistamines consumption data, loratadine and desloratadine should occur in wastewater at higher concentrations.

105 **However, loratadine has low affinity for suspended matter (octanol/water partition coefficient**  
106 **log P equal to 5 for loratadine and 3.2 for desloratadine; El-Awady et al., 2013) and therefore**  
107 **does not accumulate appreciably in sediments and remains in the water column (Moreno-**  
108 **Gonzales et al., 2015).** Loratadine is known as photolabile, in fact it is stated that the drug should  
109 be stored protected from light (Parfitt, 1999). Its UV spectrum shows an absorption band at  $\lambda$  280  
110 nm with a tail up to 300 nm, hence the drug is able to adsorb sunlight at ground level and to  
111 undergo light-induced transformations in the aquatic compartment. While its photostability has been  
112 investigated, no data on photoproducts identification are reported (Abounassif et al., 2005). In this  
113 context, we have examined the photochemical behavior of loratadine and desloratadine in aqueous  
114 medium under UVB and sunlight irradiation in order to isolate and fully characterized the  
115 photoproducts. For this purpose, concentrated solutions, far from environmental concentrations,  
116 were used (DellaGreca et al., 2014). The ecotoxicological effects of the parent drug, its metabolite  
117 and its transformation products were evaluated in producers and primary consumers.

118

## 119 **2. Materials and methods**

### 120 *2.1 Chemicals*

121 Loratadine (99.4%, CAS Number: 79794-75-5) and desloratadine (99.6%, CAS Number: 100643-  
122 71-8) were purchased by Kemprotec. All chemicals were used without further purification unless  
123 otherwise indicated. Solvents (acetonitrile, methanol and diethylether) were of HPLC grade and  
124 were purchased from Sigma Aldrich. Water was of Milli-Q quality and was obtained from a Milli-Q  
125 gradient system (Millipore).

### 126 *2.2 Apparatus*

#### 127 *2.2.1 Spectroscopic techniques*

128 Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Inova-500 instrument  
129 operating at 499.6 and 125.6 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively, and referenced with  $\text{CDCl}_3$ . The  
130 carbon multiplicity was evidenced by DEPT experiments. The proton couplings were evidenced by  
131  $^1\text{H}$ - $^1\text{H}$  COSY experiments. The heteronuclear chemical shift correlations were determined by  
132 HMQC and HMBC pulse sequences.

133 IR spectra were recorded on a Jasco FT/IR-430 instrument equipped with single reflection ATR  
134 using  $\text{CHCl}_3$  as solvent.

135 UV-Vis spectra were recorded with a Varian Cary 300 UV-Vis spectrophotometer or on a  
136 PerkinElmer Lambda 7 spectrophotometer.

#### 137 *2.2.2 Chromatographic analysis*

HPLC experiments were carried out on an Agilent 1100 HPLC system, equipped with an UV detector set at 254 nm, using a RP-18 column (Gemini, 5  $\mu$ m, 110 Å, 250 mm  $\times$  4.6 mm). at a flow rate of 0.8 mL min<sup>-1</sup>.

The analysis of the solutions used for determining the kinetic constant of loratadine photodegradation was carried out using the gradient elution as follows: at initial time 30 % acetonitrile and 70 % water for 7 min, followed by an increase of acetonitrile up to 70% in two minutes. Then, the same ratio was maintained constant for 24 minutes; finally, the initial ratio (30 % acetonitrile and 70 % water) was reached in two minutes.

In other cases HPLC analysis was performed under isocratic conditions and H<sub>2</sub>O /CH<sub>3</sub>CN 4:6 v/v was used as eluent.

GC-MS analyses were performed on a 6890 MSD quadrupole mass spectrometer (Agilent technologies) equipped with a gas chromatograph by using a Zebron ZB-5HT Inferno (5%-Phenyl-95%-Dimethylpolysiloxane) fused silica capillary column (Column 30 m  $\times$  0.32 mm  $\times$  0.10  $\mu$ m) from Phenomenex. The injection temperature was 250°C, the oven temperature was held at 50°C for 3 min and then increased to 150°C at 12°C/min, increasing to 230°C at 18°C/min, to 280°C at 10°C/min and finally to 300°C at 30°C/min and held for 3 min. Electron Ionization mass spectra were recorded by continuous quadrupole scanning at 70eV ionization energy, in the mass range of m/z 30-600.

### 2.2.3 Irradiation apparatus

The photoreactor (Multirays, Helios Italquartz) was equipped with six 15W lamps with a maximum at 310 nm (UV-B). Open quartz tubes (1 cm optical path) and open and closed pyrex tubes (20 cm  $\times$  1 cm, 25 mL) were used.

### 2.3 Chromatographic separation materials

Analytical and preparative Thin Layer Chromatography (TLC) was made on Kieselgel 60 F<sub>254</sub> plates with 0.2 mm, 0.5 or 1 mm layer thickness, respectively (Merck).

### 2.4 Experiments

#### 2.4.1 Stability in aqueous solution in the dark

Loratadine (**1**) solutions (1  $\times$  10<sup>-4</sup> M) in H<sub>2</sub>O/CH<sub>3</sub>CN (9:1, v/v) at pH 4, 7 and 9 were prepared. The acid and alkaline solutions were made using NaOH 2M and HCl 2M to adjust pH level. All solutions were kept in the dark and analyzed by HPLC (isocratic conditions) at 12 h and 48 h.

#### 2.4.2 Kinetic constant and quantum yield determination

Kinetics data were obtained by irradiating the drug (1 $\times$ 10<sup>-4</sup> M solution in H<sub>2</sub>O/CH<sub>3</sub>CN 9:1, v/v) in open quartz tubes and monitoring the solution at fixed time intervals by HPLC using the proposed gradient elution. The time evolution was fitted with a pseudo-first order equation  $C_0 = C_t \times e^{-Kt}$

172 where  $C_0$  is the initial drug concentration,  $C_t$  the concentration at time  $t$  and  $k$  the pseudo-first order  
173 degradation rate constant.

174 The incident photon flux ( $4.98 \times 10^{21}$  photons  $\text{m}^{-2} \text{s}^{-1}$ ) in solution, used to calculate the quantum  
175 yield of loratadine, was calculated using *p*-nitroanisole/pyridine actinometer (Dulin and Mill, 1982).

176 **2.4.3 Irradiation experiments.** Two solutions of loratadine in  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$  (9:1, v/v,  $1 \times 10^{-4}$  M) and  
177  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$  (75:25, v/v,  $1 \times 10^{-4}$  M) were irradiated in open quartz tube and analysed by HPLC at  
178 selected times. The photoproducts were identified by HPLC comparing their  $R_t$  values with those of  
179 standard compounds which were isolated and characterized by performing preparative  
180 photochemical experiments (see below). An aliquot of the  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$  (75:25, v/v,  $1 \times 10^{-4}$  M) was  
181 analyzed by GC-MS after 6 min irradiation.

182 **2.4.4 Preparative experiments for photoproducts isolation**

183 The photoproducts were isolated by means of preparative TLCs of irradiation mixtures obtained by  
184 appropriate experiments. Their structures were determined by spectroscopic analyses (Hesse et al.,  
185 2008). The presence of functional groups was deduced by IR spectra and identification of all  
186 different protons and carbons was obtained by NMR spectra.

187 **2.4.4.1. Isolation of isoloratadine 2**

188 Loratadine (35 mg) was dissolved in 92 mL of  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$  (75:25 v/v,  $1 \times 10^{-3}$  M) and divided in  
189 four closed quartz tubes. Each solution was saturated with argon and irradiated by UV-B lamps.  
190 After 20 min of irradiation the solvents were evaporated under vacuum and the residue was  
191 analysed by  $^1\text{H}$  NMR and separated by preparative TLC. Elution with  $\text{Et}_2\text{O}$  gave a fraction  
192 consisting of **3** and **4** (2 mg), isoloratadine **2** (6 mg), loratadine **1** (3 mg) and an intractable polar  
193 material (11 mg).

194 *Ethyl 4-(8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl)-5,6-*  
195 *dihydropyridine-1(2H)-carboxylate (2):* EI-MS  $m/z$  382/384 ;  $\text{UV}\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ) nm 266 (log  $\epsilon$  3.8);  
196  $\text{IR}\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 1690 (-N-CO-O-), 1606 (stretching vibrations of aromatic rings), 1371 (C-O  
197 stretching)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.39 (1H, d,  $J = 4.0$  Hz, H-2), 7.42 (1H, d,  $J = 7.3$   
198 Hz, H-4), 7.20-7.12 (4H, m, H-3, H-7, H-9 and H-10), 4.84-4.80 (2H, m, H-11 e H-3'), 4.10 (2H, q,  
199  $J = 7.0$  Hz,  $\text{CH}_2\text{O}$ ), 3.88 (2H, m, H-2'), 3.50-3.45 (4H, m), 2.88-2.74 (2H, m), 1.94-1.71 (2H, m),  
200 1.23 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  157.0 (C-1a), 155.5 (CO), 146.8 (C-  
201 2), 141.8 (C-6a), 138.5 (C-4), 135.9 (C-10a), 135.0 (C-4a), 133.1 (C-7), 133.0 (C-8), 131.1 (C-4'),  
202 129.8 (C-10), 126.3 (C-9), 122.4 (C-3), 121.1 (C-3'), 62.3 (C-11), 61.2 ( $\text{CH}_2\text{O}$ ), 43.4 (C-2'), 40.5  
203 (C-6'), 31.3 (x2, C-5 e C-6), 28.0 (C-5'), 14.9 ( $\text{CH}_3$ ).

204 **2.4.4.2 Isolation of compounds 3 and 4**

205 Loratadine (50 mg) was dissolved in 130 mL of H<sub>2</sub>O/CH<sub>3</sub>CN (75:25, v/v, 1 x 10<sup>-3</sup> M) and irradiated  
206 by UV-B lamps. The irradiation mixture was analyzed at different time by HPLC. After 40 min of  
207 irradiation the solvents were evaporated under vacuum, and the residue was analysed by <sup>1</sup>H NMR  
208 and separated by preparative TLC. Elution with Et<sub>2</sub>O gave a fraction consisting of **3** and **4** in ca. 3:1  
209 molar ratio (11 mg), tricycle **3** (2 mg), isolaratadine **2** (2 mg), loratadine **1** (5 mg) and an intractable  
210 polar residue (8 mg).

211 *8-Chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (3)*: EI-MS *m/z* 229/231; UVλ<sub>max</sub>  
212 (CH<sub>3</sub>OH) nm: 279 (log ε 3.1); IR ν<sub>max</sub> (CHCl<sub>3</sub>) 1580 (stretching vibrations of aromatic ring), 1070  
213 (aryl C-halogen stretching) cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.35 (1H, d, *J* = 4.9 Hz, H-2), 7.40  
214 (1H, dd, *J* = 7.5, 1.4 Hz, H-4), 7.20 (1H, d, *J* = 8.1 Hz, H-10), 7.16 (3H, m, H-4, H-7 and H-9),  
215 4.35 (2H, s, H-11), 3.33 (4H, brs, H-5 and H-6); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ 156.9 (C-1a), 146.5  
216 (C-2), 140.8 (C-6a), 137.7 (C-4), 136.0 (C-10a), 133.8 (C-4a), 132.3 (C-8), 130.7 (C-10), 128.9 (C-  
217 7), 126.3 (C-9), 122.0 (C-3), 35.7 (C-11), 31.3 (C-5 and C-6).

218 Spectral data of piperidinone **4** were deduced by those of the mixture of **3** and **4** after the signals of  
219 tricycle **3** were subtracted; it was identified by comparison of its signals with those reported in  
220 literature (Hirsch and Havinga, 1976).

221 *Ethyl 4-oxopiperidine-1-carboxylate (4)* (in mixture with **3** in ca. 1:3 molar ratio): EI-MS *m/z* 171;  
222 <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.19 (2H, q, *J* = 7.1 Hz, CH<sub>2</sub>O), 3.76 (4H, t, *J* = 6.1 Hz, H-2 and H-  
223 6), 2.45 (4H, t, *J* = 6.1 Hz, H-3 e H-5), 1.29 (3H, t, *J* = 7.1 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  
224 δ 207.1 (C-4), 155.0 (CO), 61.8 (CH<sub>2</sub>O), 43.0 (C-2 and C-6), 41.1, (C-3 and C-5), 14.6 (CH<sub>3</sub>).

#### 225 2.4.4.3. UV-B irradiation experiments for mechanistic purposes

226 Two 1 x 10<sup>-3</sup> M solutions of loratadine in pure CH<sub>3</sub>CN were prepared by dissolving 5 mg in 13 mL.  
227 A solution was irradiated in open quartz tubes and the other one in closed quartz tubes after  
228 saturating with argon. After 15 min the solvent was evaporated and each residue analysed by <sup>1</sup>H  
229 NMR.

230 A similar procedure was used for two 1 x 10<sup>-3</sup> M solutions of loratadine in methanol and for two  
231 solutions of loratadine in H<sub>2</sub>O/CH<sub>3</sub>CN (7:3 v/v).

#### 232 2.4.4.4 Irradiation of isolaratadine 2

233 A 1 x 10<sup>-4</sup> M solution of compound **2** in H<sub>2</sub>O/CH<sub>3</sub>CN (9:1 v/v) was irradiated in open quartz tubes  
234 with UV-B lamps and analysed by HPLC and <sup>1</sup>H-NMR.

#### 235 2.4.4.5 Irradiation of desloratadine 5

236 1 x 10<sup>-3</sup> M solution of desloratadine **5** was prepared by dissolving 5 mg in 16 mL of H<sub>2</sub>O/CH<sub>3</sub>CN  
237 7:3 v/v. The solution was irradiated by UV-B lamps and analysed by <sup>1</sup>H-NMR.

#### 238 2.5 Toxicity testing



239 Samples were dissolved in dimethylsulphoxide (DMSO, 3% v/v), stored in the dark at 4 °C, further  
240 diluted in deionized water (Elix 10, Millipore, Milan, Italy) and sonicated for 30 minutes to obtain  
241 stock solutions. The test solutions were prepared by mixing the appropriate volumes of the stock  
242 solutions and ISO test media. Toxicity assays were performed in the following organisms: the green  
243 alga *Pseudokirchneriella subcapitata*, the planktonic rotifer *Brachionus calyciflorus* abundant in  
244 freshwaters, the anostracan crustacean *Thamnocephalus platyurus*, highly sensitive in acute toxicity  
245 testing and the cladoceran crustacean *Ceriodaphnia dubia*, worldwide distributed and often  
246 employed in acute and chronic toxicity testing.

#### 247 2.5.1 Determination of drugs concentration in test samples

248 The concentrations of drugs were **measured (n=1)** using the solid phase extraction (SPE) coupled  
249 with HPLC. Each test solution containing drugs at the beginning of each toxicity test and after 24 h,  
250 48 h and 72h passed through a C18 Sep-Pak® light column (Waters) used as a solid phase extraction  
251 cartridge, previously conditioned with 5.0 mL methanol followed by 5.0 mL water. The cartridge was  
252 then eluted with 5 mL methanol. The eluate was evaporated to dryness under reduced pressure and  
253 the residue was suspended in 1.0 mL acetonitrile. Portions of 200 µL volume were then injected into  
254 the HPLC system.

#### 255 2.5.2 Acute toxicity tests

256 *B. calyciflorus* organisms were hatched from cysts (MicroBioTest Inc., Nazareth, Belgium) in  
257 synthetic moderately hard freshwater (80-100mg/L CaCO<sub>3</sub>, pH7.5 ± 0.3) at 25 ± 1 °C under  
258 continuous illumination (3000-4000 lux) for 16-18 h prior to test initiation, as reported in the  
259 ASTM E 1440-91 guidelines. Six replicates with five animals/well, less than 2h old, were  
260 performed for each concentrations (0.3mL of test solution for each test well in 36-well plates,  
261 MicroBioTest Inc., Nazareth, Belgium) of each compound.

262 The *T. platyurus* test was performed in according to ISO 14380 (2011) using larvae hatched from  
263 cysts (Thamnotoxkit F, MicroBioTest Inc., Nazareth, Belgium) in 20-22 h before the assay in the  
264 standard freshwater (dilution 1:8 with deionized water) at 25°C under continuous illumination  
265 (3000-4000 lux). Tests were performed in 24-well plates with 10 crustaceans/well (1.0 mL of test  
266 solution), in three replicates.

267 The *C. dubia* test was performed over 24 h of exposure using young organisms less than 24 h old  
268 following test conditions reported in EPA-600-4-90 (US EPA 1993) with slight modifications.  
269 Neonates of at least third generation coming from a healthy mass culture (starting organisms were  
270 purchased from Aquatic Research Organisms, Inc., Hampton, NH, USA) were maintained at 25 ± 1  
271 °C in synthetic medium (hardness 250 mg/L expressed as CaCO<sub>3</sub>) with a 16:8 h light:dark cycle

(600 lux) Tests were performed in 24-well plates with 10 crustaceans per well (1.0 mL of test solution), in three replicates.

For each test considered above, both a negative control (only test-medium) and a solvent control (DMSO 1% v/v related to the maximum concentration of compounds tested equal to 100 mg/L) were performed. The plates were incubated in darkness at 25 °C for 24 h.

The end-point considered was mortality, and the concentration resulting in a 50% effect in 24 h-exposure was indicated as Median Lethal Concentration (LC50).

In acute assays, compounds were tested for a maximum of eight dilutions depending on the respective sensitivity of the organisms (100-31.25-9.76-3.15-0.98-0.31-0.09 mg/L) starting from the highest concentration of 100 mg/L with a geometric progression of 3.2.

### 2.5.3 Chronic toxicity tests

The *B. calyciflorus* chronic test was based on the offspring reduction over 48 h exposure (ISO, 20666, 2008) and was performed on young organisms less than 2 h old. Cysts were hatched as previously described for the acute test. Tests were performed in 48-well plates with one rotifer/well (0.9 mL of test solution prepared in moderately hard dilution water, ASTM E1440-91), in six replicates. The organisms were fed with a fresh suspension (0.1 mL) of  $10^7$  cells/mL of the unicellular alga *P. subcapitata*. Plates were incubated in darkness at 25 °C.

The chronic test in *C. dubia* was performed with female neonates < 24 h old from at least the third generation of a stock culture maintained in synthetic water with ISO medium were individually exposed to 25 mL of test solution in beakers over 7 days (ISO, 20665, 2008). Tests were conducted in semi-static conditions (all test media were exchanged five times per week) and, from the fourth day-exposure onward, the offspring produced by each parent organism were counted and removed daily. The organisms were fed daily with 200 µL of a combination of yeast *Saccharomyces cerevisiae*, alfalfa and flake food in addition to the unicellular green alga *P. subcapitata* ( $10^8$  cells/mL). Ten replicates per concentration were incubated at  $25 \pm 1$  °C with a 16:8 h light:dark cycle (600 lux).

The *P. subcapitata* growth inhibition test was performed according to OECD 201, 2011 with slight modifications reported by Paixao et al., 2008. The single samples were incubated with  $10^4$  cells/mL of algal suspension in 96-well microplates in six replicates under continuous illumination at  $25 \pm 1$  °C on a microplate shaker (450 rpm). The plates were read at 450 nm (SpectraFluor, Tecan, Switzerland) immediately before the test and after 24 h, 48 h and 72 h.

For all chronic tests, a negative control (test-medium control) was used to the test series. Only for *P. subcapitata* growth inhibition test, the % DMSO exceeded the maximum % recommended in toxicity testing (0.01%). Thus, for this kind of assay, a solvent control (DMSO 0.1% v/v related to

the maximum concentration of compounds tested equal to 10000 µg/L) was performed. The number of the offspring or the algal growth outputs were compared to the values obtained for the negative control in order to determine the chronic effective percentages and to evaluate the chronic Effective Concentrations (ECx).

In *B. calyciflorus* and *C. dubia* chronic assays, compounds were tested for a maximum of nine dilutions (1000-312.5-97.66-30.52-9.54-2.98-0.93-0.29-0.09 µg/L) starting from the highest concentrations of 1000 µg/L with a geometric progression of 3.2. For the *P. subcapitata* growth inhibition test, compounds were tested for a maximum of ten dilutions (10000-3125-976.56-305.17-95.37-29.80-9.31-2.91-0.91-0.28 µg/L) starting from the highest concentration of 10000 µg/L with a geometric progression of 3.2.

#### 2.5.4 Ecotoxicological data analysis

For each kind of assay, three independent experiments were performed. For each independent experiment, the effect percentages were calculated comparing each specific negative control. For each assay, the effect percentages coming from three independent experiments were pooled using Prism5 software (Graphpad Inc., CA, USA) to estimate the concentrations giving x% effect (L(E)Cx) by non-linear regression (log agonist vs. normalized response-variable slope). The LC50 value, corresponding to the 50% of mortality for each test-organism, was the test parameter for acute tests, whereas EC50, EC20, and EC10 were the concentrations giving 50%, 20% or 10% of the effect used in chronic tests to evaluate the inhibition of reproduction or the inhibition of the algal growth.

### 3. Results

**The SPE coupled with HPLC analysis** revealed a non-appreciable difference between nominal and actual concentrations: the actual concentrations of tested chemicals **diverged from the nominal concentrations by 5% after 24h, around 10% after 48h, and around 15% after 72h.** According to Li (2012), when the actual concentrations are at least 80% of the nominal concentrations, the measured and the expected concentrations are considered to be very close and no significantly different, so that in the present study the effective concentrations are reported as nominal concentrations.

#### 3.1 Photochemical behaviour of loratadine

Loratadine **1** is slightly soluble in water, hence acetonitrile was chosen as co-solvent to obtain clear solutions (Figure 1). Preliminary experiments were carried out in the dark using  $1 \times 10^{-4}$  M solutions in H<sub>2</sub>O/CH<sub>3</sub>CN 9:1 v/v. The drug was stable after 48 h even when tested in acidic (pH 4)

340 and alkaline (pH 9) solutions. These pH ranges are usually considered in environmental analysis  
341 (Valenti et al., 2009).

342 Loratadine solution was then irradiated in a photoreactor with UV-B lamps. HPLC analysis  
343 showed the formation of photoproducts already after 2 min (Figure 2): compound **2** at  $R_t$  16.2 min  
344 and compound **3** at  $R_t$  10.2 min (Figure 1). The photoproducts were identified comparing their  $R_t$   
345 values with those of standard compounds which were isolated and characterized by performing  
346 preparative photochemical experiments.

347 Kinetic experiment under these conditions showed that loratadine has a half-life of 137.4  
348 seconds and a polychromatic quantum yield of  $5.89 \times 10^{-4}$  (Table S1).

349 Preparative experiments to isolate and characterize the photoproducts were carried out by UV-B  
350 irradiation of  $1 \times 10^{-3}$  M solutions of the drug in  $H_2O/CH_3CN$  75:25 v/v. HPLC analysis confirmed  
351 the trend observed in dilute conditions and revealed the presence of the peaks of products **2** and **3**  
352 together with other minor products. After 40 min of irradiation, TLC on silica gel afforded three  
353 photoproducts: compounds **2** and **3** and a new product **4**. Structures **2-4** (Figure 1) were assigned on  
354 the basis of spectral data. In particular, 1D and 2D NMR spectroscopy was used because it is a  
355 powerful technique for identification and structure elucidation of small organic molecules  
356 (Elyashberg, 2015; Fuloria and Fuloria, 2013).

357 The mass spectrum of photoproduct **2** ( $R_t$  16.5 min) shows a molecular peak at 382/384  $m/z$   
358 corresponding to the molecular formula  $C_{22}H_{23}ClN_2O_2$ , hence suggesting that it is a loratadine  
359 isomer. The mass spectrum evidences a peak at  $m/z$  154, absent in the mass spectrum of loratadine,  
360 attributable to the tetrapyridine fragment  $C_8H_{12}NO_2$ . The  $^1H$ -NMR spectrum shows significant  
361 differences only in the aliphatic proton region. In particular, two overlapping signals due to protons  
362 H-11 and H-3' (singlet + multiplet, respectively) are observed at  $\delta$  4.80 as expected due to the shift  
363 of the double bond. The shift of the double bond produces, in the  $^{13}C$ -NMR spectrum, the  
364 disappearance of the singlet carbon signal at  $\delta$  133.3 (C-11 of **1**) and the appearance of a doublet  
365 carbon signal at  $\delta$  120.8 (C-3' of **2**).

366 The structure of photoproduct **3** ( $R_t$  10.2 min) was confirmed by the presence in the mass spectrum  
367 of molecular peak at 229/231  $m/z$  corresponding to the molecular formula  $C_{14}H_{12}ClN_2$ . The  $^1H$ -  
368 NMR spectrum shows the presence of six aromatic protons in the  $\delta$  range 8.40-7.10, of signals at  $\delta$   
369 4.35 and at  $\delta$  3.33 due to di-benzylic methylene proton H-11 and to benzylic methylene protons H-5  
370 and H-6, respectively.

371 Piperidinone **4** was obtained by TLC in mixture with compound **3** (ca. 1:3 molar ratio) and its data  
372 were deduced by comparison with those reported in literature (Hirsch and Havinga, 1976). It was  
373 not observed by HPLC analysis since it is transparent at the selected wavelength (254 nm) of the

detector. Its presence in the irradiation mixture was confirmed by GC-MS (Figure 2) and <sup>1</sup>H-NMR analysis of the crude irradiation mixture.

NMR analysis was particularly useful to examine the irradiation mixtures since loratadine and its photoproducts **2-4** have characteristic identifiable signals.

Aqueous solutions of the drug were also exposed to sunlight in Naples (Italy) in July 2017, under environmental-like conditions. As expected, degradation was slower. HPLC analysis showed a decrease of drug concentration to approximately 50% after 2 days and a complex mixture of photoproducts. The chromatographic and spectroscopic analysis of the irradiation mixture showed the presence of photoproducts **2-4**.

### 3.2 Mechanistic interpretation

In order to gain more mechanistic information on photoproducts formation, UV-B irradiation experiments were performed under various conditions (in different solvents such as acetonitrile, methanol, water/acetonitrile; in the presence and absence of oxygen) and the reactions were monitored by HPLC and <sup>1</sup>H NMR. The experimental conditions and the results are reported in Table S2.

Accordingly with previous data (Abounassif et al., 2005), loratadine degraded faster in solutions containing water (after 15 min of irradiation only 5% photodegradation in methanol or acetonitrile vs. 55% in H<sub>2</sub>O/CH<sub>3</sub>CN 7:3 v/v, Table S2 runs **c**, **e**, **g**). In all irradiation conditions, especially under argon, photoproduct **2** was present while the formation of compounds **3** and **4** was observed only in aqueous solution. Control experiments showed that isoloratadine **2** was photolabile and converted to unidentified material after 20 min of UV-B irradiation.

On the basis of literature data, a plausible mechanistic interpretation is reported in Figure 3. Compound **2** should derive from a 1,3-hydrogen shift, probably via a radical pair, from an excited triplet state of loratadine **1**, as suggested by the quenching with oxygen. The radical recombination can give loratadine **1** or its isomer **2** (Turro et al., 2010a). Addition of water to give intermediate **6** and β-cleavage of the alkoxy radical intermediate **7** should give products **3** and **4**. β-Cleavage of alkoxy radicals to give ketones and stable radicals is well known (Turro et al., 2010b).

In all the experimental irradiation conditions, desloratadine **5** was not observed. This result is not surprising considering that the carbamate function is quite photostable and it does not absorb light in the UV-C and UV-B regions (Iesce et al., 2006). However, control experiments showed that when a 1 × 10<sup>-3</sup> M solution of desloratadine **5** in H<sub>2</sub>O/CH<sub>3</sub>CN 7:3 v/v was irradiated by UV-B lamps as reported above for loratadine, it was photodegraded within 60 min and gave a complex mixture of products among which the sole identifiable product was tricycle **3**.

### 408 3.3 Ecotoxicological experiments

409 Tests were performed with loratadine **1**, and its photoisomer **2** and the mixture of compounds **3** and  
410 **4** (in ca. 3: 1 molar ratio) obtained by preparative experiments (see 2.4.4). We also examined  
411 desloratadine **5** and its photodegradation mixture (DPM) obtained as reported in 2.4.4.5.

### 412 3.4 Acute toxicity results

413 In order to verify that the acute effects were not DMSO-dependent, a solvent control was performed  
414 for each kind of assay, at the highest tested percentage (1% v/v), and referred to the highest tested  
415 concentration of 100 mg/L, observing no significant difference with the negative control, with a  
416 survival higher than the 90% both in negative and in solvent controls (Table S3) as recommended  
417 by test validity criteria. The parent compound loratadine, its metabolite desloratadine, the  
418 transformation products and the degradation mixture of desloratadine were found to cause mortality  
419 in both crustaceans and rotifers and the LC50 values, obtained after 24 h exposure (coming from  
420 three independent experiments pooled using Prism5) are reported in Table 1. In addition, LC50  
421 values expressed as mean  $\pm$  SD of the independent experiments are reported in Table S4. Loratadine  
422 was able to cause the 50% of mortality in *C. dubia* at hundreds of  $\mu$ g/L, differently from the effects  
423 found at dozens of mg/L in the rotifers and in the anostracan crustacean. On the other hand, all the  
424 aquatic organisms showed the same sensitivity to the metabolite, with LC50 values found at units of  
425 mg/L, while DPM was more active in the rotifer *B. calyciflorus* (LC50 equal to 2.02 mg/L) than in  
426 crustaceans. Transformation products such as isoloratadine **2** and the mixture of tricycle **3** and  
427 piperidinone **4** showed different acute effects. In fact, albeit isoloratadine was more effective in *C.*  
428 *dubia* (LC50= 1.19 mg/L), the mixture of tricycle **3** and piperidinone **4** caused 50% mortality at  
429 units of mg/L not only in the cladoceran crustacean but also in the rotifer and it is more lethal than  
430 parent loratadine **1** for *B. calyciflorus* (Table 1). To the best of our knowledge, scientific data on the  
431 aquatic toxicity of the compounds here tested is rather scarce. **Nevertheless, there are several data**  
432 **on diphenhydramine (DPH), the same histamine H1-receptor antagonist as loratadine.** In 2013  
433 Goolsby and collaborators found that the diphenhydramine (DPH) was acutely toxic in *C. dubia*  
434 with an LC50 value equal to 3.94 mg/L, while in 2015 Kristofco et al., found that DPH caused a  
435 50% immobilisation in the cladoceran crustacean *Daphnia magna* at 374  $\mu$ g/L after 48h-exposure  
436 and the 50% mortality in the fish *Danio rerio* at 45.5 mg/L after 72h exposure. In the 2011,  
437 Berninger et al. found a median acute effect in *D. magna* at 0.37 mg/L after DPH exposure and  
438 from units to dozens of mg/L in the fish *Pimephales promelas*. Differently from vertebrates which  
439 are known to possess some degree of genetic homology for DPH targets like histamine-H1  
440 receptors, with a similarity from 40 to 70% (Gunnarsson et al., 2008, Berninger et al., 2011), the  
441 effects of the antihistamines in invertebrates may likely be related to other mechanisms of actions

442 affecting histamine ion channel transporters, as suggested by Haas et al., 2008 and Berninger et al.,  
443 2011. Regarding transformation products, they were found to be slightly toxic for all aquatic  
444 organisms tested excepted isoloratadine 2 in *C. dubia*. **Although acute toxicity data are generally**  
445 **very far from those of environmental concern and from the water solubility of chemicals, they**  
446 **are still relevant regarding the assessment of environmental risk since chronic data are often**  
447 **lacking.**

### 448 3.5 Chronic toxicity results

449 The chronic toxicity data for the five samples, reported as EC50, EC20, and EC10 values (coming  
450 from three independent experiments pooled using Prism5) and expressed in  $\mu\text{g/L}$ , are shown in  
451 Table 2. In addition, EC50 values expressed as mean  $\pm$  SD of the independent experiments are  
452 reported in Table S5. In order to verify that the chronic effects were not DMSO-dependent, a  
453 solvent control was performed only for *P. subcapitata* growth inhibition assay, at the highest tested  
454 percentage (0.1% v/v), and referred to the highest tested concentration of 10000  $\mu\text{g/L}$ , as explained  
455 above. No significant difference with the negative control was found, with a growth higher than the  
456 90% both in negative and in solvent controls (Table S3) as recommended by test validity criteria.  
457 Loratadine 1 was the most chronically active compound in the rotifer (EC50= 51.32  $\text{mg/L}$ ), while  
458 its metabolite desloratadine 5 was the most active both in *C. dubia*, with a median effective  
459 concentration at few units of  $\mu\text{g/L}$ , and in the green alga *P. subcapitata* (EC50 = 220.20  $\mu\text{g/L}$ ), as  
460 also depicted in Figure 4, where the concentration/effect curves of samples are reported for each  
461 aquatic organism. As shown in Figure 4, the parent compound was less active in *C. dubia* than its  
462 metabolite but more active than the transformation products. Furthermore, DPM determined a  
463 median chronic toxic effect at hundreds or thousands  $\mu\text{g/L}$ , in the case of aquatic consumers and  
464 producers, respectively (Table 2). In fact, as also reported in Figure 4, differently from consumers  
465 (*C. dubia* and *B. calyciflorus*), in producers (*P. subcapitata*) there is a slow increase in  
466 concentration/effect relationship with an evident response only at the highest concentrations.

467 In 2015 Watanabe and collaborators tested the diphenhydramine histamine H1-receptor antagonist  
468 in the alga *P. subcapitata* finding an EC50 value equal to 1240  $\mu\text{g/L}$ , the same order of magnitude  
469 of the EC50 obtained in this study.

470 In the 2009, Isidori and collaborators tested the ranitidine, a histamine H2-receptor antagonist in the  
471 consumers *C. dubia* and *B. calyciflorus* finding a median offspring reduction in the order of  
472 thousands of  $\mu\text{g/L}$ , therefore underlining a higher sensitivity of these aquatic organisms to  
473 histamine H1- than to histamine H2-receptor antagonists. The environmental chronic toxicity of the  
474 histamine H1-receptor antagonist such as loratadine and its derivatives towards the aquatic tested  
475 organisms is observable already at few units-dozens  $\mu\text{g/L}$  (EC20 values, Table 2), however these

476 residues occur in surface waters at ng/L levels which are too low to cause an immediate threat to  
477 exposed organisms but can pose delayed long term effects interfering with organism metabolic  
478 pathways.

479 **The EC10 and EC20 values of loratadine and EC10, EC20 and EC50 values of desloratadine**  
480 **(Table 2) are lower than their respective water solubility equal to 11 µg/L and 3950 µg/L,**  
481 **making the results of this study interesting to understand the behaviour of these drugs in real**  
482 **water samples. At the best of our knowledge, no data of photoproducts water solubility is**  
483 **available.**

#### 484 **4. Conclusion**

485 Loratadine **1** is transformed either under UVB or by sunlight exposure. The reactive site is the  
486 double bond while the carbamate moiety is unreactive. Transformation products derive by  
487 photoisomerization and water photoaddition followed by a cleavage reaction. Photolability is also  
488 observed in desloratadine **5** but this drug leads to a complex photodegradation mixture.

489 The toxic effects of loratadine **1** and desloratadine **5** occur in both acute and chronic assays at  
490 concentrations higher than their environmental occurring concentrations differently affecting the  
491 organisms selected from two trophic levels. However, the environmental transformations of the  
492 parent compounds, here simulated by the UV-irradiation treatments, lead to the formation of a  
493 bioactive mixture of residues and transformation products, which could represent a harmful  
494 combination to some of the organisms tested. **In order to define water quality criteria protective**  
495 **for all aquatic species, further toxicity studies towards other aquatic species are needed**  
496 **especially to increase species sensitivity diagrams used in EU and North American approach**  
497 **to anti-histamine management to derive water quality benchmark, and to broaden knowledges**  
498 **of mechanisms involved in the different biological responses of the organisms.**

499

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504

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